National Toxicology Program
Toxicity Report Series
Number 73

NTP Report on the Metabolism, Toxicity, and Predicted Carcinogenicity of

Diazoaminobenzene

(CAS No. 136-35-6)

September 2002

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Toxicity Study Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals.

These studies are designed and conducted to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Toxicity Study Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's toxic potential.

Details about ongoing and completed NTP studies are available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov. Abstracts of all NTP Toxicity Study Reports and full versions of the most recent reports and other publications are available from the NIEHS' Environmental Health Perspectives (EHP) http://ehp.niehs.nih.gov (800-315-3010 or 919-541-3841). In addition, printed copies of these reports are available from EHP as supplies last. A listing of all the NTP Toxicity Study Reports printed since 1991 appears on the inside back cover.

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NTP Report
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(CAS No. 136-35-6)

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CONTENTS

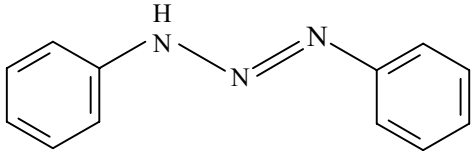
SUMMARY

Background: Diazoaminobenzene is used as a laboratory reagent and occurs as an impurity in cosmetics, food products, and pharmaceuticals. The structure of the chemical is a combination of benzene and aniline, both of which are known to cause cancer. We performed tests to determine if diazoaminobenzene might pose a similar hazard.

Methods: We exposed male and female rats and male mice to single doses of diazoaminobenzene (applied on the skin, injected into the blood, or inserted directly into the stomach through a tube) to determine if the chemical breaks down into benzene or aniline in the body. We also applied diazoaminobenzene to the skin of male and female rats and mice for 16 days to determine its pattern of toxicity.

Results: We found benzene, aniline, and their breakdown products (metabolites) in the blood of rats within 15 minutes after dosing with diazoaminobenzene. Benzene was detected in the breath of rats and mice, and all the metabolites in the urine were the same as those known to result from benzene and aniline in rats and mice. In the 16-day study, some toxic effects associated with aniline (methemoglobinemia) and with benzene (atrophy of the lymphoid tissue) occurred in rodents administered diazoaminobenzene.

Conclusions: Diazoaminobenzene is converted to the known carcinogens aniline and benzene and produces similar toxic effects as those two chemicals. Based on these results, we predict that diazoaminobenzene is also a carcinogen.



DIAZOAMINOBENZENE

CAS No. 136-35-6

Chemical Formula: $C_{12}H_{11}N_3$ Molecular Weight: 197.24

Synonyms: Anilinoazobenzene; benzeneazoanilide; benzeneazoaniline; DAAB; alpha-diazoamidobenzol; p-diazoaminobenzene; 1,3-diphenyltriazene; 1,3-diphenyl-1-triazene; DPT; N-(phenylazo)aniline
Trade names: Cellofor; Porofor DB

ABSTRACT

Diazoaminobenzene is used as an intermediate, complexing agent, and polymer additive. It is also an impurity in certain color additives used in cosmetics, food products, and pharmaceuticals. Diazoaminobenzene was selected for metabolism and toxicity studies based on the potential for worker exposure from its use in laboratories, positive *Salmonella typhimurium* gene mutation data, its presence as an impurity in foods and cosmetics, and the lack of adequate toxicity data. Several structural analogues and presumed metabolites of diazoaminobenzene are carcinogenic, providing evidence for the possible carcinogenicity of diazoaminobenzene. The chemical structure of diazoaminobenzene suggested that it would be metabolized into aniline and benzene; therefore, metabolism and disposition studies were performed in male and female F344/N rats and male B6C3F$_1$ mice administered a single oral, dermal, or intravenous dose of diazoaminobenzene. Electron spin resonance (ESR) studies were conducted to assess the possible formation of a phenyl radical from the reduction of diazoaminobenzene by components of the cytochrome P450 mixed-function oxidase (P450) system in microsomes or by gut microflora in anaerobic cecal incubations. Bile duct-cannulated male F344/N rats were administered diazoaminobenzene and 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) for *in vivo* determination of the DMPO-phenyl radical. 16-Day toxicity studies were performed to identify target organs of diazoaminobenzene following dermal application to male and female F344/N rats and B6C3F$_1$ mice.

In the disposition and metabolism studies, oral doses of 20 mg/kg to male and female rats and male mice were readily absorbed and excreted mainly in the urine, with exhalation of volatile organics accounting for about 1% of the dose. The only volatile metabolite detected in the breath was benzene, and all the metabolites in the urine were those previously shown to result from the metabolism of benzene and aniline in rats and mice. While dermal doses to rats

and mice (2 and 20 mg/cm^2) were only slightly absorbed, benzene and aniline metabolites were nonetheless detected in the urine. High circulating levels of benzene, aniline, and their metabolites were detected in the blood of rats administered 20 mg/kg diazoaminobenzene as early as 15 minutes after exposure. At 24 hours after dosing, diazoaminobenzene was detected at low levels (<1%) in the adipose tissue, blood, kidney, liver, muscle, skin, and spleen. Metabolites of benzene and aniline were also formed in an *in vitro* study using human liver slices.

In the ESR spin-trapping experiments, the ESR spectrum of the DMPO-phenyl radical was detected when diazoaminobenzene was incubated with microsomes or P450 reductase, DMPO, and NADPH, or when incubated with cecal contents and DMPO. The DMPO-phenyl radical spectrum was not attenuated by the P450 inhibitor, 1-aminobenzotriazole, or carbon monoxide suggesting that P450s were not required. In *in vivo* experiments in which rats were administered diazoaminobenzene and DMPO, the DMPO-phenyl radical adduct was detected in bile within 1 hour after treatment.

In the 16-day toxicity studies, groups of five male and five female F344/N rats and B6C3F$_1$ mice received dermal applications of 0, 12.5, 25, 50, 100, or 200 mg diazoaminobenzene/kg body weight. Animals were evaluated for absolute and relative organ weights, for hematological effects, and for gross and microscopic lesions. No mortality occurred in rats. However, most male mice exposed to concentrations of 50 mg/kg or greater and female mice exposed to 200 mg/kg died. Body weights of male and female rats and female mice were less than those of the vehicle controls. Similar chemical-related toxicities were observed in both species. Clinical pathology data indicated a chemical-related methemoglobinemia and Heinz body formation in male and female rats and mice. Analysis of organ weights indicated possible chemical-related effects in the thymus, heart, spleen, kidney, and liver of rats and/or mice. Increases in the incidences of several skin lesions, including hyperplasia of the epidermis and hair follicles, and inflammation in rats and mice and ulceration in female mice were observed. Other nonneoplastic lesions that were considered to be related to diazoaminobenzene administration were atrophy of the thymus, mandibular and/or mesenteric lymph nodes, and white pulp of the spleen, as well as splenic hematopoietic cell proliferation in rats and mice. In mice, there were increased incidences of atrial thrombosis, and necrosis was observed in the renal tubules and liver.

Diazoaminobenzene was mutagenic in *S. typhimurium* strains TA98, TA100, and TA1537 with induced rat or hamster liver S9 enzymes; no activity was noted in strain TA1535, with or without S9. *In vivo*, two gavage administrations of either diazoaminobenzene or benzene induced highly significant increases in micronucleated polychromatic erythrocytes in bone marrow of male B6C3F$_1$ mice at all doses tested.

Diazoaminobenzene is metabolized to the known carcinogens benzene and aniline. Further evidence of this metabolism is that some toxic effects associated with aniline (methemoglobinemia) and benzene (atrophy of the lymphoid tissue) were identified. Based on these results, it is predicted that diazoaminobenzene is a carcinogen.

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies of diazoaminobenzene on October 18, 2001, are listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that this Toxicity Study Report presents the experimental results and conclusions fully and clearly. The comments of the reviewers were received and reviewed prior to the finalization of this document. Changes have been made such that the concerns of the reviewers have been addressed to the extent possible.

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SUMMARY OF PEER REVIEW COMMENTS

On October 18, 2001, the draft Technical Report on the toxicity studies of diazoaminobenzene received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Committee and associated Peer Review Panel. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. N.B. Ress, NIEHS, introduced the report on the metabolism, toxicity, and predicted carcinogenicity of diazoaminobenzene by describing the study design process and the results of metabolism and disposition studies and 16-day dermal toxicity studies. She also described results of a mouse bone marrow micronucleus study (not presented in the draft report) that showed that diazoaminobenzene, like benzene, is a potent inducer of micronuclei. The proposed conclusions to the report were:

> Diazoaminobenzene is metabolized to the known carcinogens benzene and aniline. Some toxic effects associated with aniline (Heinz body anemia, methemoglobinemia) and benzene (atrophy of the lymphoid tissue, hematopoietic cell proliferation) were identified. Based on these results, it is predicted that diazoaminobenzene is a carcinogen.

Dr. Thrall, a principal reviewer, agreed with the prediction of carcinogenicity based on the metabolism of diazoaminobenzene to benzene and aniline but questioned whether Heinz body anemia was truly an effect, as only one of 20 treated groups had a statistically significant increase in Heinz body formation. She asked for clarification of whether oral exposure occurred during the dermal study and suggested rearranging the conclusion statement to clarify that the prediction of carcinogenicity was based on metabolism. Dr. Ress replied that some oral exposure occurs in dermal studies as a result of the animals grooming themselves. In these studies the animals were housed individually to minimize such exposure.

Dr. Klaunig, the second principal reviewer, was unable to attend the meeting and his comments were read into the record by Dr. M.S. Wolfe, NIEHS. Dr. Klaunig agreed that the study results supported the premise that diazoaminobenzene may be carcinogenic.

Dr. Piegorsch, the third principal reviewer, agreed with the conclusions and asked if the results on the micronucleus studies would be included in the final version of the report. Dr. J.R. Bucher, NIEHS, indicated that the micronucleus data would be added with the understanding that these data were not used by the review panel in formulating the conclusion statement.

Dr. Hecht asked if phenyl hydrazine would also have been an expected metabolite of the compound and if any consideration had been given to possible interactive effects between the metabolites benzene and aniline. Dr. Ress replied that while phenyl hydrazine could be a metabolite, it was not observed in these studies. The possibility of interactive effects between the metabolites was being examined in further micronucleus tests.

Dr. Thrall moved that the conclusions be modified to eliminate mention of Heinz body anemia and hematopoietic cell proliferation. The revised conclusion was:

> Diazoaminobenzene is metabolized to the known carcinogens benzene and aniline. Further evidence of this metabolism is that some toxic effects associated with aniline (methemoglobinemia) and benzene (atrophy of the lymphoid tissue) were identified. Based on these results, it is predicted that diazoaminobenzene is a carcinogen.

Dr. Piegorsch seconded the motion, which was approved unanimously with five votes.